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ORIGINAL ARTICLE

An accurate and reliable method for identification and quantification of fatty acids and trans fatty acids in food fats samples using gas chromatography

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Abstract A method for the separation, identification and further quantification of fatty acids (FAs) and trans fatty acids (TFAs) by gas chromatography (GC) using the combination of lipid extraction and derivatization with the base-catalysed method followed by trimethylsilyl-diazomethane (TMS-DM) was developed. The proposed method was found to allow sensitive and accurate determination of a wide range of different types of FAs, including TFA isomers. The method was validated on real samples of dietary fat from hydrogenated edible oils (margarine) and nine standard FAs as representatives of margarines. For this purpose, response linearity, limit of detection (LOD), limit of quantification (LOQ), precision and recovery (*R*%) were all determined. Based on the results obtained, *R*-values from all the samples were revealed to be close to 100%, repeatability RSD ranged between 0.89% and 2.34%, and reproducibility RSD values ranged between 1.46% and 3.72%. The applicability of this method was demonstrated in four margarine samples and it was compared with the method used as reference. In general, the results proved that the proposed method is suitable for the analysis of FAs since it has shown higher effectiveness in TFA analysis than the classic methods. Thus, it could be an effective tool for analysing dietary fats and oils in complex mixtures of food products for the monitoring of low levels of FAs and TFA, and the control of labelling authenticity.

1. Introduction

Oils and fats available in nature are found in the form of triglycerides (TRG). The FA composition of TRG has a direct effect on the physical and chemical properties of edible fats and oils (Bockisch, 1998). Solid fat contains a high proportion of saturated FAs (SFA); while oils are often in the liquid state as they contain a high proportion of monounsaturated FA (MUFA) and/or polyunsaturated FA (PUFA) (Dixit and

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Das, 2012; Waheed et al., 2009). Naturally, all unsaturated FAs in the plant oils are in the cis-form (Salimon et al., 2012). A small percentage of unsaturated FAs isomerises to their TFA counterparts during the extraction, refining and deodorization processes or during the heating and frying of oils at high temperatures, while the proportion increases during industrial hydrogenation of oils (Choe and Min, 2007; Kemény et al., 2001). Food products made with hydrogenated fats and oils such as margarines, shortenings, confectionery fats, bakery products and others also contain TFA (Aro et al., 1998; Bhanger and Anwar, 2004; Tarrago-Trani et al., 2006).

The results obtained in many previous studies indicate the importance of food FA composition in human nutrition and health (Simopoulos, 2002; Williams, 2000). In contrast, hydrogenated fats and oils prevent rancidity and are used in foods to improve texture and stability for a longer shelf life because TFA have higher melting points and greater stability than their cis isomers (Alonso et al., 2000; Wassell and Young, 2007). However, unfortunately, several clinical studies published in the last few years have indicated that the intake of TFA may be a risk factor for cardiovascular disease by raising serum cholesterol levels in low-density lipoproteins, and, in addition, that there are no known nutritional benefits over other fats (Backholer and Peeters, 2012; Kummerow, 2009). Over the past few years, it has been pointed out that this association between dietary consumption of some FAs and increased risk of some diseases has led to the implementation of new regulations requiring the declaration of FAs including TFA content on the labels of conventional foods and dietary supplements in several countries (Brandt et al., 2009). However, the ability to report the TFA content of a food as "0," meaning less than 0.5 or 0.2 g per serving (according to the US and Canadian regulations, respectively), requires knowledge of the minimum amount that can be accurately quantified (Mossoba et al., 2007; Tyburczy et al., 2012). Consequently, accurate methods for measurement of FAs and TFAs with adequate sensitivity and precision are needed to achieve those objectives.

Although several analytical methods, such as Ag^{+}-HPLC, Infrared spectroscopy and capillary electrophoresis have been developed over the last decade, GC coupled with mass spectrometer or flame ionization detector (FID) is the most widely used technique for analysing essential oils and food fats (Al-Qudah, 2011; Basconcillo and McCarry, 2008; Christie and Han, 2010; Jumat et al., 2006). Commonly, most methodologies used for determining FAs are lipid extraction followed by conversion of the FAs into corresponding methyl esters (FAMEs) (Brondz, 2002; Delmonte et al., 2009). Such methodologies are usually used for preparing FAMEs from lipids either by basic hydrolysis followed by methylation of the free FAs or by transesterification of lipids using acid or base catalysed as rapid and simple methods (Alrouh et al., 2012; Delmonte and Rader, 2007). However, each of these procedures has their own advantages and disadvantages (Basconcillo and McCarry, 2008).

Recently, these methods have been optimized for the analysis of specific foods (Phillips et al., 2010). However, variable and inaccurate results are possible when analysing products having a complex mixture of FAs and TFA even under optimal conditions of column length, stationary phase and operating parameters, due to the chemical instability of PUFA and the changing distribution of cis and trans isomers entering GC (Palmquist and Jenkins, 2003; Ratnayake and Beare-Rogers, 1990). Moreover, to obtain accurate quantitative results, it is necessary to address potential procedural difficulties, such as incomplete conversion of the FAs into FAME, formation of artefacts and contamination, alterations of the original FA profile during esterification (positional and/or geometrical isomers, i.e., TFA isomers) and subsequent damage of GC column (Ackman, 1998; Palmquist and Jenkins, 2003; Rozema et al., 2008).

On the other hand, food fats, such as margarines, consist mainly of triacylglycerol molecules with little non-lipid contaminants (Christie, 1993; Marais, 2007). The presence of non-lipids at the moment of the derivatization process may lead to interference with lipids and that will cause potential errors with high variable profiles (Christie and Han, 2010; Harmanescu, 2012; Juárez et al., 2008). Thus, the isolation of all the lipids in the sample in their native state must be accomplished before being analysed (Marais, 2007). Nevertheless, few papers deal with lipid extraction in depth.

According to Juàrez et al. (2008) the alkali-based transesterification method has resulted in poor recoveries of FAMEs because free FAs might remain partially unreacted. Otherwise, the methylation of FFAs using TMS-DM, after the saponification process, has been reported to be more accurate for PUFA analysis in seafood and conjugated linoleic acid isomers in ruminant meat tissues compared to other methylation reagents such as acidic catalyses (Aldai et al., 2005; Juárez et al., 2010). However, the hydrolysis or presence of traces of water also leads to poor recoveries of FAMEs (Basconcillo and McCarry, 2008). Consequently, it is possible to apply the advantages of sodium methoxide ($NaOCH_3$) as a useful reagent for fast transformation of FAs into FAMEs (Christie, 1993) along with using TMS-DM reagent for a complete methylation of all FFAs.

This method could provide an analysis and quantification of a complex mixture of FAs and TFA in a single chromatogram and overcome the problems affecting the accuracy when monitoring low levels of TFA in dietary fats and oils or their products, such as margarine samples and blended fats, in order to correct nutrition labelling and control the labelling authenticity. The objective of this study is to develop an accurate method for GC analysis of FAs and TFA from oils and fats using the extraction of lipid and methylation with $NaOCH_3$ followed by TMS-DM. Moreover, it aims to apply the proposed method to analyse the FA of margarine samples in order to validate this method and demonstrate its applicability for determining the FA in different rich samples in saturated, mono-, poly-unsaturated with special emphasis on TFA.

2. Experimental

2.1. Standards, reagents and samples

Nine standards of FAs were selected based on their different chain length, geometric structure (cis and trans) and double bond positions. They are identified as Lauric (C12:0), Myristic (C14:0), Palmitic (C16:0), Stearic (C18:0), Oleic (C18:1), Elaidic (C18:1t9), Linoleic (C18:2), Linolelaidic (C18:2t 9, 12) and Alpha-Linolenic acids (C18:3). All nine standards of the FAs as well as the internal standard (IS) C15:0 (Pentadecanoic acid) were purchased from Sigma (Sigma–Aldrich, Germany)

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and the purity was at least 99%. The individual FAMEs were purchased from Fluka (purity; ⩾99% (GC); Sigma–Aldrich, Germany). All solvents and reagents were of an analytical grade, specially for chromatography and purchased from Systerm (Systerm, Malaysia) except n-hexane, which was of a higher purity grade (Systerm, Malaysia, for GC, ⩾99%). The esterifying agent TMS-DM (2 M) in n-hexane was purchased from Sigma (Sigma–Aldrich, Germany).

Four different samples of vegetable oil-based margarine samples were selected for the analysis and validation of the proposed method. The samples were purchased from several Malaysian local supermarkets and were coded with these numbers (1, 2, 3 and 4), respectively.

2.2. Calibration and standard preparation

A standard mixture containing all FAs, as shown in Table 1, was used to prepare the stock solution (Std1). Calibration curves were produced from five working standard sets Std2–Std6, which were prepared daily from Std1 by diluting half a volume with n-hexane. The stock solution of the internal standard was prepared by dissolving 0.1 g pentadecanoic acid (C15:0) in 10 mL hexane. All the solutions of the working standards and IS were stored at −20 °C until analysis.

2.3. Extraction and preparation of samples

Before derivatization, the FA constituents of the margarines samples were processed by accurately weighing 150 mg aliquots of the homogenised samples into extraction tubes. Lipid extracts were prepared by homogenising the samples in 20 ml of hexane containing (50 ppm) *teri*-butylated hydroxy toluene (BHT) to avoid oxidation of cis and trans PUFAs. The homogenates were dried with anhydrous sodium sulphate Na_2SO_4 and filtered with sintered glass funnels. After washing the funnels with 5 ml of hexane, the solvent was removed under vacuum in a rotary evaporator. Finally, the extracted lipids of margarine samples were dried under nitrogen, weighed carefully and stored frozen until analysis.

2.4. Preparation of fatty acid methyl esters (FAMEs)

After the extraction process, FAMEs were prepared before the GC analysis. For this, each of extracted lipids of margarine samples was transferred to a screw-cap test tube (10 mL) and 1 mL of a solution containing 10 mg 5 mL^{-1} heptadecanoic acid (internal standard) in methanol was added and followed by 1 mL of 2 M $NaOCH_3$. The content was placed in a water bath at 60 °C for 5 min. Drops of concentrated glacial acetic acid were added to each tube to neutralize NaOH. The samples were reduced to dryness under N_2 and then redissolved in 1 mL of methanol: toluene (2:1 vol.). After that, TMS-DM was added in molar excess of 2 M in n-hexane (100 μl) at 50 °C for 10 min without capping the tubes. Drops of glacial acetic acid were added until the yellow colour disappeared to remove unreacted TMS-DM and the reaction mixture was diluted with 1 ml of 0.5% NaCl solution. To extract the FAME, 1 mL of n-hexane containing 50 ppm BHT was added and the tubes were vortexed for about 30 s. After the solution settled, the organic layers, containing the methyl esters, were transferred to a vial for GC.

2.5. Gas chromatographic analysis

One microlitre volume of each sample was injected into GC (Shimadzu, GC-17A, Kyoto, Japan) equipped with FID for separation and quantification of the FAMEs. The analysis was carried out using a BPX-70 fused silica capillary column (30 M, 0.25 mm i.d., 0.2 μm film thickness, Melbourne, Australia). The run was under an optimised temperature programme as follows: initial column temperature 100 °C, programmed to increase at a rate of 10 °C min^{-1} up to 160 °C and then at 3 °C min^{-1} up to 220 °C. This temperature was maintained for 5 min, then at 10 °C min^{-1} up to final temperature of 260 °C and held for 5 min. Injector and detector temperatures were at 260 and 280 °C, respectively. Helium was used as the carrier gas at a flow rate of 1 mL min^{-1} with a split ratio of 30:1.

2.6. The general measurement procedures

2.6.1. Identification and calibration

The standard solutions and samples were submitted to a similar procedure. The working standard sets (Std2–Std6) were derivatized, as previously described, and injected (1 μL) five times in the GC under the same conditions used for analysing the samples. FAMEs in margarine oil samples were identified by conducting a comparison of similar peak retention times (Rt) using pure FA standards. Calibration curves were constructed from the analyses in triplicate of the aforementioned working standards for quantitative purposes. According to the Multiple Point I.S. method, the calibration plot of each

Table 1 The concentration of fatty acid mixture standard in stock solution.

Fatty acids	Formula	Concentration in (std1) (mg/ml)
Lauric acid	C12:0	8
Myristic acid	C14:0	10
Palmitic acid	C16:0	100
Stearic acid	C18:0	30
Elaidic acid	C18:1tran-9	15
Oleic acid	C18:1	120
Linolelaidic Acid	C18:2 trans-9,12	12
Linoleic acid	C18:2	50
Linolenic acids	C18:3	25

Std1, stock solution.

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compound was run by applying the ratio of the peak area of the FAME in the standards to the peak area of the IS against the ratio of the concentration of the FAME to the concentration of the IS. The concentration of FAME in the margarine oil was then determined using the area ratio and the calibration plot. The composition of the FAs in the margarine oil was then recalculated and determined as w/w percentages and expressed as mg FA 100 g^{-1}. For the validation of the quantitative method, response linearity of the pure FAs, detection and quantification limits, robustness, recovery, and precision of the analytical procedure were all calculated.

2.6.2. Validation procedure

The method was subject to validation according to the guidelines for validation of chromatographic methods (Taverniers et al., 2004; Wood et al., 1998). The validation procedure included detector response linearity and sensitivity, limit of detection and quantification, recovery and precision assays.

Linearity was determined from the values of correlation coefficient (r) obtained from calibration curves with a range of concentrations as previously reported, and the sensitivity of the detector was determined from the values of slope obtained from linear regression equations for each analyte.

The limit of detection (LOD) and limit of quantification (LOQ) of the proposed method were determined from the calibration curves of FA standard and the mean noise value as the mean area of the signal obtained on analysing six blanks. The LOD for the chromatographic peaks was expressed as peaks with an area at least three times the mean noise and LOQ as peaks with an area of at least ten times the mean noise.

The precision of the method was checked through the repeatability and reproducibility experiment. The repeatability of the method was calculated by using the measured data of a single day, and the reproducibility of the method was calculated by using the measured data of three successive days. Both values were expressed by relative standard deviation (RSD%).

For the recovery of the method, it was established by spiking a margarine sample with a standard working solution at three concentrations (Std2, Std4, Std6), and assaying it in triplicate. The concentrations of the FAs in the nonspiked margarine were subtracted from the concentrations in the spiked margarine and the recovery percentages (R%) were calculated by dividing the calculated concentrations by the expected concentrations.

2.7. Statistical analysis

A paired t-test was used to compare differences between mean values for the content of each FA measured using the proposed method and the method used as standard (significance level $P \leqslant 0.05$). The calculation of means, standard deviations and RSD% were performed using Microsoft® Excel (Professional Edition 2007; Microsoft Corporation, Redmond, WA).

3. Results and discussion

3.1. Analysis of margarine samples

Four margarine samples varying in FAs and TFA content were analysed in triplicate in order to validate and demonstrate the applicability of the proposed method. Fig 1 shows a typical FID chromatogram of total FA analysis on margarine (sample 1) obtained using the proposed procedure as previously outlined.

As can be observed, the baseline separation was achieved for all components within 35 min with a good separation between FAs and TFA peaks, thus, indicating that the peak overlapping was not interfered with by the peaks of major constituents as opposed to the chromatograms presented by a routine analysis (Christie, 1993; Phillips et al., 2010). Furthermore, no strange peaks or artefacts interfering with the FA chromatographic peaks were found. This result also confirms what was earlier reported that TMS-DM did not produce any methoxy artifacts associated with base-catalysed (Aldai et al., 2005; Juárez et al., 2008, 2010; Murrieta et al., 2003; Yamasaki et al., 1999).

The concentration of all 9 FAs studied, including TFAs, was analysed and calculated for all four margarine samples in absolute (g/100 g) and relative (w/w percentages) content. Table 2 presents the mean of the absolute (g FA 100 g^{-1} sample) and relative (% of total identified FA) content for each FA determined using the proposed method.

3.2. Response linearity and sensitivity

The tested FA standards were detected as FAMEs by GC-FID in approximately a 35 min single run analysis with a good separation between peaks. Table 3 presents the retention time (Rt), linearity ranges, the equation and correlation coefficients (r) of the calibration curves for the different pure FAs selected.

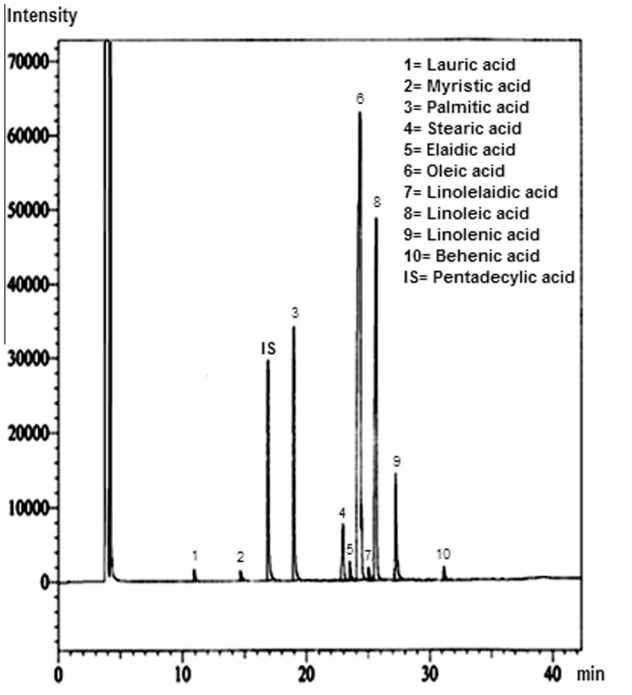

Figure 1 GC-FID chromatogram of margarine (Sample1). 1 = Lauric acid; 2 = Myristic acid; 3 = Palmitic acid; 4 = Stearic acid; 5 = Elaidic acid; 6 = Oleic acid; 7 = Linolelaidic acid; 8 = Linoleic acid; 9 = Linolenic acid; 10 = Behenic acid; IS = Pentadecylic acid.

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Table 2 Absolute (g FA 100 g^{-1} sample) and relative concentrations (% of total identified FA) of FAs in four margarine samples.

Fatty acids	Detected concentration [g FA 100 g^{-1}], (% Total FA)			
	Sample 1	Sample 2	Sample 3	Sample 4
C12:0	[0.28], (0.34)	[0.12], (0.15)	[0.11], (0.13)	[0.07], (0.12)
C14:0	[0.39], (0.46)	[0.72], (0.89)	[0.43], (0.51)	[0.14], (0.25)
C16:0	[10.77], (12.96)	[36.85], (45.50)	[27.49], (32.73)	[7.42], (13.26)
C18:0	[2.70], (3.26)	[3.74], (4.62)	[1.95], (2.32)	[2.02], (3.61)
C18:1 tran-9	[0.64], (0.77)	[0.15], (0.19)	[1.50], (1.78)	[0.17], (0.31)
C18:1	[42.87], (51.58)	[31.86], (39.34)	[26.65], (31.73)	[32.27], (57.62)
C18:2 trans-9,12	[0.47], (0.56)	[0.10], (0.12)	[0.82], (0.98)	[0.13], (0.24)
C18:2	[20.34], (24.48)	[6.82], (8.42)	[21.92], (26.10)	[10.64], (19.01)
C18:3	[4.05], (4.87)	[0.32], (0.4)	[1.59], (1.90)	[2.25], (4.03)
Others	(0.33)	(0.25)	(1.82)	(1.54)

Table 3 Linearity and estimated regression parameters for FA standard obtained from calibration curves.

Fatty acids	Retention time (R_t)	Linear ranges (Std2-Std6) (mg/ml)	Calibration Curve Equation [a]	r
C12:0	10.954	4–0.25	y = 0.1866 x + 0.0262	0.9963
C14:0	15.060	5–0.313	y = 0.3049 x + 0.0008	0.9931
C16:0	19.613	50–3.125	y = 0.2477 x + 0.0076	0.9955
C18:0	24.102	15–0.938	y = 0.3494 x + 0.0034	0.9900
C18:1 tran-9	24.591	7.5–0.47	y = 0.3607 x + 0.0051	0.9972
C18:1	24.916	60–3.75	y = 0.3431 x + 0.0060	0.9947
C18:2 trans-9,12	25.555	6–0.375	y = 0.2914 x + 0.0006	0.9960
C18:2	26.375	25–1.563	y = 0.1789 x + 0.0063	0.9976
C18:3	28.077	12.5–0.781	y = 0.2792 x + 0.0121	0.9963

Std, standard solutions; r: coefficient of correlation.
[a] y = FA peak area/IS peak area, x = [FA]/[IS], IS – internal standard (C15:0).

Separate calibration plots were used to calculate the linear response of the FAs in the FID detector.

According to the linear regression equations obtained from calibration curves, the results were found to be linear over the concentration range studied and the coefficient of correlation values was higher than 0.99 for all the FAs studied. The slope values of the regression lines for all FAs were almost similar (mean value of 0.271), which is indicative of similar detector calibration sensitivity for each analyte (Slemr et al., 2004).

3.3. Limit of detection and quantification

The results of LOD and LOQ were calculated from the mean noise value by analysing six blanks, and established by multiplying the mean noise value by 3 and 10, respectively. The results of the calculations of LOD and LOQ for the different pure FAs considered are shown in Table 4. The range of LOD values obtained was from 0.03 to 0.09 μg mL^{-1} (0.3–0.9 ng, respectively), and the range of LOQ was from 0.1 to 0.2 μg mL^{-1} (1 and 2 ng, respectively) for the target FAs.

Based on the observation of the data, oleic acid (C18:1) and palmitic acid (C16:0) obtained the highest values for LOD (0.09 and 0.08 mg mL^{-1}, respectively) and also for LOQ (0.2 and 0.18, respectively), while the lowest values for both limits (0.03 and 0.1 mg mL^{-1}, respectively) were obtained by C12:0 and C18:2 t9, t12. This is probably due to the higher concentration of C18:1 and C16:0, and lower concentration of C12:0 and C18:2 C18:2 t9, t12 in a stock solution. In addition, the values of both limits in other FAs were intermediate. Overall, both limits are lower than those obtained for these analytes by other authors (Buchgraber and Ulberth, 2002; Delmonte and Rader, 2007; Mossoba et al., 2007; Tyburczy et al., 2012).

3.4. Robustness

Robustness of the method was evaluated in three different experiments. For the first experiment sample weight was varied in the range of 120–180 mg. Obtained results for FA composition in the tested sample confirmed that a slight change in sample weight does not have any influence on the results. In the next experiment the conditions of the GC analysis

Table 4 Intervals of limit of detection and limit of quantification of ranged values.

Fatty acids	LOD (μg/ml)	LOQ (μg/ml)
C12:0	0.03	0.10
C14:0	0.04	0.12
C16:0	0.08	0.18
C18:0	0.05	0.12
C18:1 tran-9	0.05	0.10
C18:1	0.09	0.20
C18:2 trans-9,12	0.03	0.10
C18:2	0.07	0.16
C18:3	0.05	0.14

LOD, limit of detection; LOQ, limit of quantification.

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Table 5 The repeatability (RSD%) of the FAs determined in margarine samples.

Fatty acid	Sample (n = 6, RSD%)[a]			
	1	2	3	4
C12:0	1.90	2.21	1.62	1.50
C14:0	1.71	1.42	1.23	2.40
C16:0	0.89	1.26	1.43	1.03
C18:0	2.12	1.63	1.29	1.49
C18:1 tran-9	1.53	1.39	1.40	1.11
C18:1	1.15	1.21	2.10	1.52
C18:2 trans-9,12	1.84	2.34	1.16	1.73
C18:2	0.95	1.18	1.93	1.58
C18:3	1.26	1.59	0.97	1.67

[a] RSD, relative standard deviation.

Table 6 The reproducibility (RSD%) of the FAs determined in margarine samples.

Fatty acids	Sample (n = 3, RSD%)[a]			
	1	2	3	4
C12:0	2.40	2.58	1.95	2.43
C14:0	3.21	2.91	2.60	3.04
C16:0	2.14	2.14	2.05	2.60
C18:0	2.58	2.94	2.88	2.37
C18:1 tran-9	2.03	3.52	3.23	2.51
C18:1	3.44	3.43	3.10	1.57
C18:2 trans-9,12	1.84	3.19	2.41	2.67
C18:2	2.06	2.60	1.77	1.62
C18:3	2.58	2.67	2.42	2.99

[a] RSD, relative standard deviation.

(temperature programme and carrier gas flow) were changed (80–120% of prescribed conditions). Resolution of all peaks was slightly different in experiments than in chromatograms obtained by the prescribed conditions but in the acceptable range. The robustness of experiments proves that it is possible to increase the recovery by using the hexane for washing the samples two times after derivatization process without significantly losing the produced FAMEs although this was not done by reason of routine sample methylation (Society and Firestone, 1998).

3.5. Precision of the method

The repeatability and reproducibility replications on real samples (margarines) were used to measure the precision of the quantitative method. The repeatability of the method was established from six (n = 6) complete analyses of each sample under the same conditions in a day, and the reproducibility was established from three (n = 3) complete analyses of each sample repeated for three consecutive days. The repeatability and reproducibility data are shown in Table 5 and Table 6, respectively, and the results expressed as a relative standard deviation (RSD,%).

It was observed that the repeatability ranged between 0.89% and 2.34% for all target analytes, and the reproducibility ranged between 1.46% and 3.72%, with an average value of 1.52% and 2.45%, respectively. Thus, these values can be considered as acceptably accurate and the obtained results confirm the precision of this method because the RSD values are more consistent and lower than those values obtained from the improved standard methods (Antolin et al., 2008; Phillips et al., 2010; Rozema et al., 2008; Xu et al., 2010).

3.6. Accuracy

The accuracy of the proposed method was verified by means of a recovery assay. The recovery percentage of the method was established from the complete analysis in triplicate of margarine samples fortified with the selected working standard (std2, std4, std6). The recovery percentages (measured concentrations/real concentration × 100) obtained for each FA studied are shown in Table 7.

In general, the mean of R values ranged from 93.8% to 104.5% for all spiked concentrations and all the samples. They increased when the spiked standard decreased from Std2 to

Table 7 Recovery factor (R%) at three addition levels for the four studied margarines.

Sample	Std	Fatty acids (R%, n = 9)[a]								
		C12:0	C14:0	C16:0	C18:0	C18:1 t9	C18:1 t9, t12	C18:2	C18:2	C18:3
1	2	97.1	94.2	96.1	94.5	96.2	95.8	94.9	98.2	99.5
	4	99.2	97.9	97.2	95.3	97.7	99.1	97.1	101.8	103.0
	6	102.7	97.3	98.7	96.1	103.3	97.6	102.2	102.9	104.1
2	2	95.5	96.2	94.3	97.5	96.2	94.1	101.7	99.8	98.1
	4	98.2	101.3	97.9	94.3	94.7	95.8	102.1	100.8	101.0
	6	103.7	98.4	99.3	98.8	104.1	99.0	102.6	102.5	104.6
3	2	98.1	96.8	96.1	96.5	93.9	97.1	94.0	98.2	104.0
	4	99.9	95.4	97.2	97.8	97.7	98.9	97.1	101.6	102.3
	6	103.4	97.3	97.7	99.1	104.1	100.6	104.2	102.9	103.7
4	2	96.5	95.8	97.3	101.4	100.4	94.1	102.7	100.4	101.5
	4	98.4	94.3	97.4	96.7	98.1	95.8	101.1	102.4	103.1
	6	101.7	99.6	100.6	98.0	98.8	99.0	103.4	104.5	104.1

[a] R, recovery; Std, standard solution; t, trans fatty.

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Std6. PUFA showed the highest R values (average of 101.9%) in comparison to other FAs (average of 99.5% for TFA and 97.1% for SFA). The highest variation in the *R* values for TFA was observed in sample 3 with 10% higher R values in Std6 than in Std2 probably because this sample contained the lowest percentage of TFA.

Accordingly, *R*-values that were approaching 100% indicated that the proposed method is appropriate in terms of accuracy, credibility, and more reliable for a quantified analysis of a mixture of FAs and TFAs in fats and oils and their dietary products than other methods (Phillips et al., 2010; Schreiner, 2005; Stolyhwo and Rutkowska, 2012; Tyburczy et al., 2012).

3.7. Application of the method

In order to demonstrate the applicability of the proposed method, it was used to determine the FAs and TFA in food fats and oils through four margarine samples (containing mixtures of vegetable oils, partially hydrogenated oils and oils fraction) as it was previously described in Section 3.1.

In addition, concentrations of FAs obtained by using the proposed method were compared with those FA concentrations obtained through the method (Society and Firestone, 1998) used as standard. A paired *t*-test was used to compare the concentrations of FAs obtained by each method. The results ($p > 0.05$) showed that there were no statistically significant differences between the concentrations obtained by the two compared methods.

4. Conclusions

The proposed GC method based on the combination of lipid extraction and derivatization using $NaOCH_3$ followed by TMS-DM allows the determination of free and esterified FAs in different food fats and oils with a high degree of accuracy and reliability. The method also allows the separation and identification of cis/trans isomers due to the effect of these isomers on the health of consumers. This derivatization method yields satisfactory results on preparing FAMEs from FAs for GC analysis, including TFA, without having to make auxiliary separations and also without changing the original isomer distribution or geometric configuration of TFA. Moreover, the lack of artefacts interfering with the FA chromatographic peaks provides sufficient evidence to suggest that the proposed methylation method is a suitable alternative for the analysis of a complex mixture of FAs spanning a broad range of molecular types, as found in hydrogenated vegetable oils and blended fats. Accordingly, it could be recommended that the application of this method can be used as an effective tool if it is applied for analysing FAs and TFAs in different food samples such as margarine products, shortenings, fat frying, baking industry and bakery products for correct nutrition labelling and control of labelling authenticity.

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